ORIGINAL

SURFACE MODIFICATION FOR BIOCOMPATIBILITY

Contract # NS 5-2322

Quarterly Progress Report #11

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The University of Michigan

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Quarterly Progress to:	National Institutes of Health
Contract Monitor:	William Heetderks, Ph.D.
Research Contract:	"Surface Modification for Biocompatibility"
Contract #	NS 5-2322
Principal Investigators	David C. Martin and K. Sue O'Shea
Date:	October 31, 1997

Overview

This report is a summary of research carried out in our laboratories during the third quarter of 1997. It is organized by subgroup: Martin lab, O'Shea lab, Kresge group.

1. Martin Laboratory

Probes were coated for two groups of external users. Dr. Andrew Schwartz was sent the 2 x 8 probe array coated with SLPL solution (200 mg/ml) and aqueous NGF (1 mg/ml) in 8:1 proportions. To coat only the tips of the probes, a slit was made in a piece of parafilm and the ends of the array were exposed through the slit. The contact was made using carbon tape from the electrode to the connectors at the base of the array. It deposited on the exposed ends and some down the outer teflon coating. This is visible in the 'after' images as the fibers are just under the resolution of the optics. Figures One and Two illustrate probes before coating. Note the teflon coating goes all the way to the end of the electrodes, the stainless wire is only exposed at the end. Figures Three and Four illustrate probes after coating. The fibers are barely visible, but probes were well coated even as visualized using an optical microscope.

Probes were also coated for the Messinger lab. Three types of probes were coated for implantation into toadfish. Probes (n=47) were coated with and without NGF. The procedure for coating consisted of spinning 150 mg/ml solution of SLPL with and without NGF onto the probes. The probes were unmounted silicon, and were placed on a silicon wafer chip approximately 1 cm square. A glass coverslip was laid over the probe shafts to expose only the tip for coating. Coating was done at a 2 cm height, at approximately 6000 V for two seconds. A typical probe end is shown in Figure Five.

2. O'Shea Laboratory

As we reported previously, using standard lithographic technologies, Dr. Libby Louie was able to produce patterned substrates with three very critical characteristics. The pattern consisted of lanes of stripes of protein interspersed with bare glass. At the end of each was a 90^{o} turn, incorporated to insure that cells followed the adsorbed protein rather than just streaming from

the center. These protein stripes varied in thickness (2, 10, 50 micrometers), and interstripe distance was also varied (2, 10, 50 micrometers). These variables were (somewhat unexpectedly) important in determining substrate-specific behavior. Finally, we incorporated a protein free zone at the center of the radiating spokes, to contain cells at the beginning of the culture period. Libby also found that by careful pipetting, she was able to coat individual spokes with a single protein, so that a culture well contained a variety of substrate choices. Figure Six illustrates the patterned substrate.

Work in the last few months in the O'Shea lab has focused on the *in vitro* analysis of cell behavior on these patterned substrates. Neuronal (Neuro 2A) and glial cells were grown on coverslips patterned with native proteins (fibronectin and laminin) or with biopolymer containing the RGD cell recognition sequence of fibronectin (SLPF) or the IKVAV neurite outgrowth promoting sequence from laminin (SLPL). Neuro 2A growth cones were compact on all substrates examined (Figures 7-9), microspikes were present on all proteins, and large veils were observed on SLPL. Glial cell growth cones were most elaborate on fibronectin coated substrates; less spread on SLPF, laminin or SLPL (Figures 10-12). Mags = 680X.

At least six replicate cultures have been carried out for each cell type on each substrate, and we are currently analyzing growth parameters including: distance migrated, cell substrate association, characteristics of the neuronal and glial cell leading process. Preliminary observations suggest that neuronal cells migrate more rapidly than glial cells regardless of substrate, that cells do not respond differently to biopolymers v native protein substrates, with the exception of glial cells on SLPL, where growth cone behavior was more fibronectin like than laminin like.

In terms of patterning and cell behavior, a number of interesting observations have resulted from these experiments. Firstly, neuronal cells respect the protein glass boundaries, and distinguish between substrates. Neuronal cell morphology varied strikingly with protein stripe thickness (Figure 13 A,B). Neurons, unlike glial cells, were able to adhere to 2 micrometer thick stripes of protein.

Glial cells were able to over-bridge regions of glass to reach adjacent protein stripes, and while they spread on all substrates examined, their morphology was substrate specific. Glial cells were not able to adhere to 2 micrometer stripes, unless the stripes were close together, enabling the cell to bridge them, and "read" the substrate as adhesive. Cells plated on thin stripes were unable to attach and underwent apoptosis. It will be of particular interest to determine the nature of cell - substrate interaction of cells on varying stripe thickness using interference reflection microscopy (IRM) currently being developed in our lab.

We also carried out preliminary analyses of signalling molecules present in the basal cytoplasm. A summer student, Carla Kovacs, carried out localization of focal adhesion kinase (FAK), protein tyrosine kinase, and paxillin. We were unable to demonstrate major differences in the distribution of these molecules using this approach, and are planning to use IRM to assess cell-substrate interactions in the next round of experiments. Carla presented her data at a meeting at the University of Michigan in August, and has returned to her undergraduate pre-med program at Harvard.

Current investigations are in progress to quantify observations of cell migration (distance) on the various protein substrates (native and artificial), to extend these to SEM and IRM, and begin to prepare a manuscript for submission.

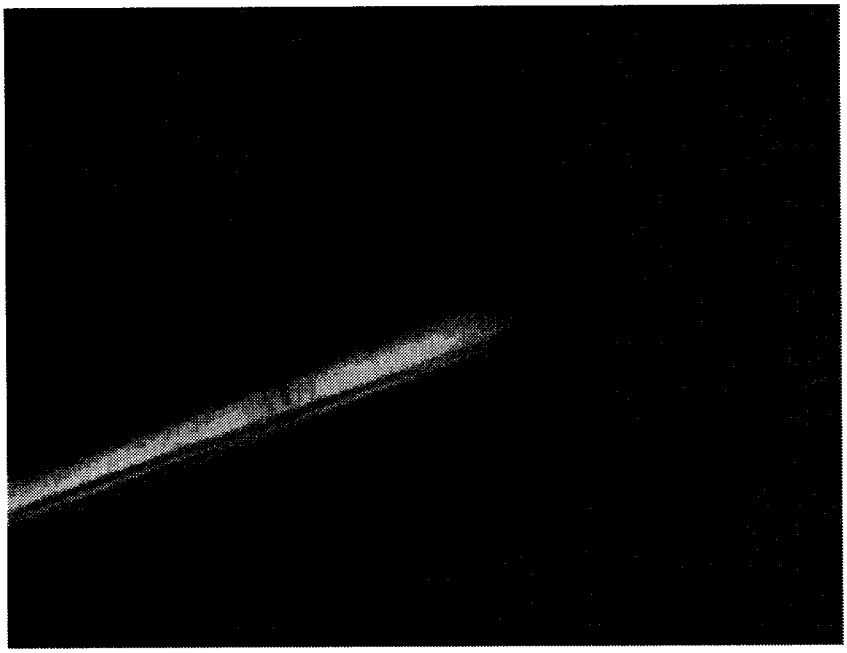
3. Kresge Group

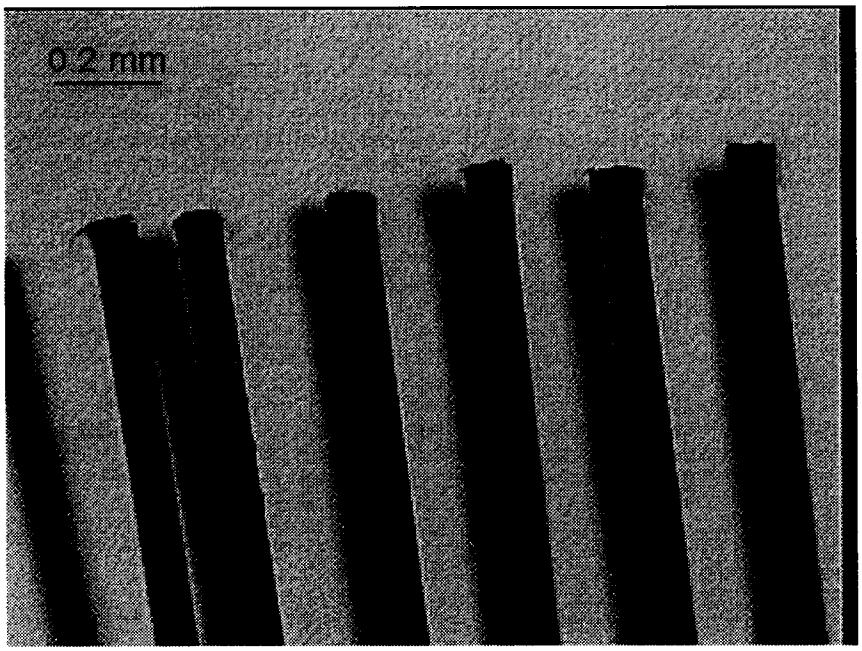
The Kresge Hearing Research Institute investigators have made considerable progress in our ability to image the probe - tissue interface. Briefly, coated 3-D probes were implanted into Guinea pig cortex and after three weeks, animals were perfused, tissue blocked and exposed to propidium iodide (to allow visualization of cell nuclei). Blocks are infiltrated with a modified low viscosity resin, Spurr's. Blocks are cut with a diamond saw, polished and the first 50-100 micrometers of tissue containing the silicon probe imaged using the rhodamine channel on our Biorad laser confocal microscope.

To date, we have examined uncoated and SLPF coated probes. In both there are areas of nuclei accumulation at the edge of the probe. Figure 14 illustrates laser sections through an uncoated (A,B), and an SLPF coated (C,D) probe illustrating the range in cell re-organization at the probe surface. Occasionally, this appearance will involve 3-7 electrode shafts over a 10-30 micrometer depth, with other regions unaffected. Clearly, all the embedded probes (n=9) must be analyzed throughout their lengths before valid conclusions can be made. These studies are ongoing.

Future Directions

We would like very much to extend our observations to more quantitative analysis of brain - probe adhesion, to examine "floating" probes, to examine c-fos (a marker of neuronal activity) expression in these brains. I would particularly like to do the fos work, as it would allow us to determine precisely how far from the implant neurons were affected. However, given time and funding constraints, it is likely that our work will focus on analysis of existing data, specifically cell behavior on patterned substrates and laser confocal analysis of implanted, coated probes.




0.2 mm

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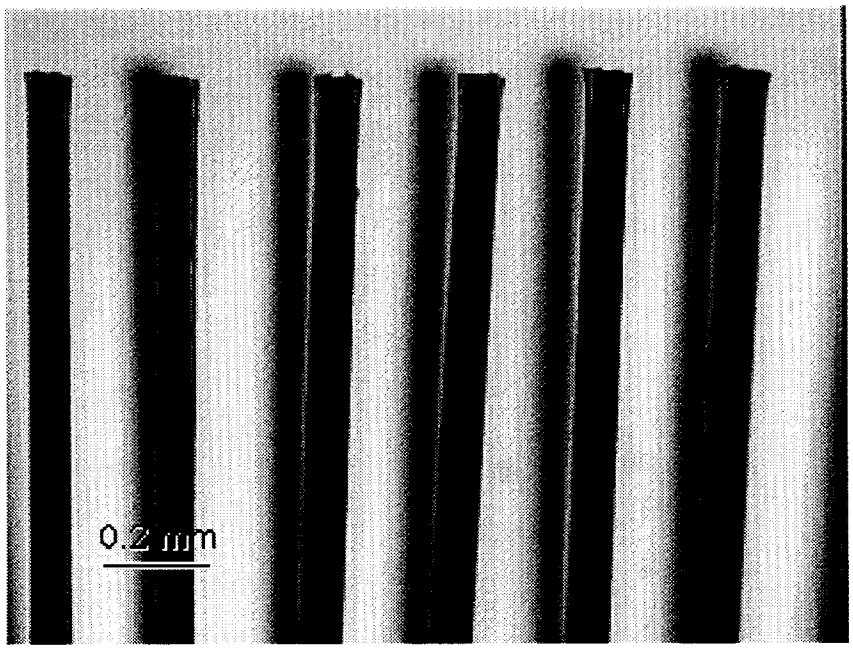

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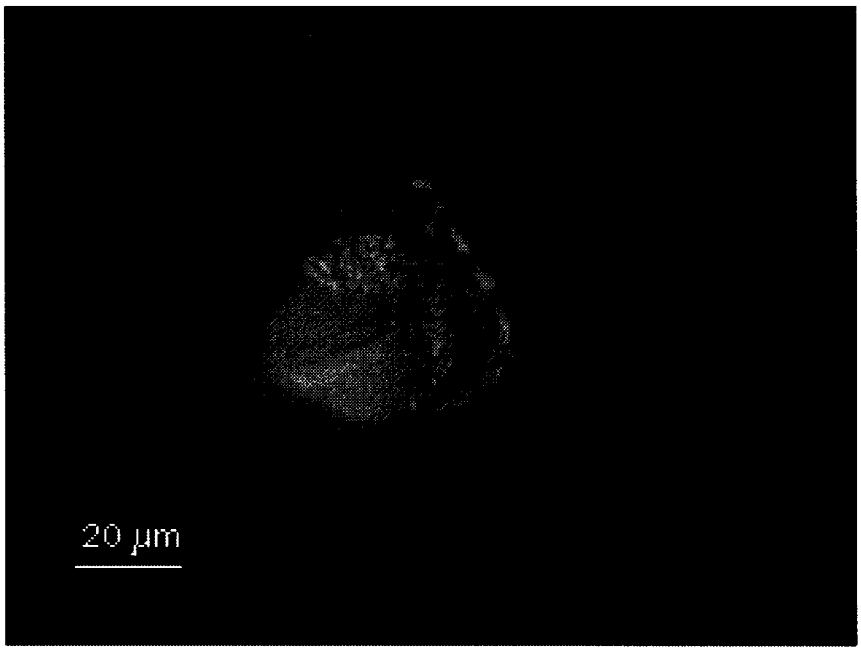
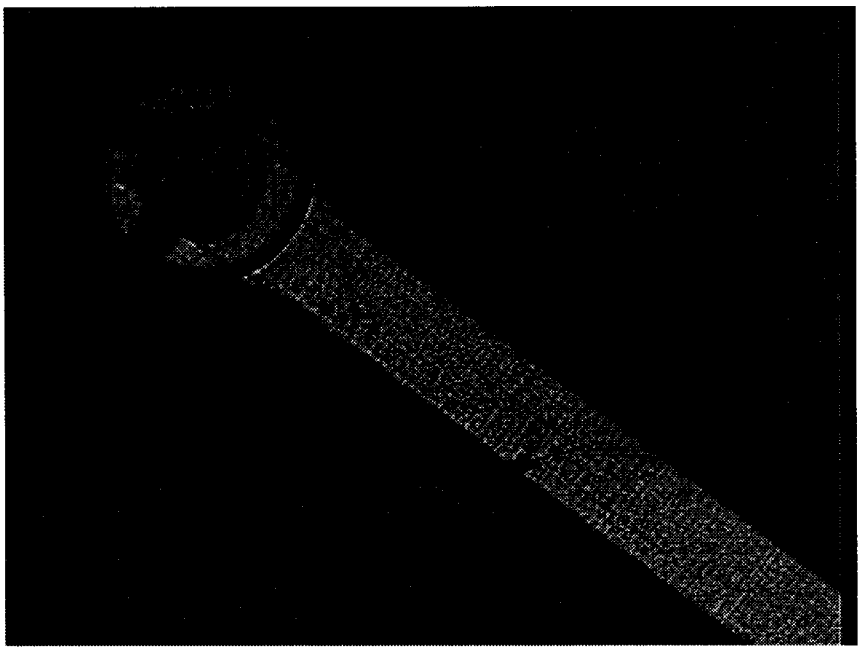
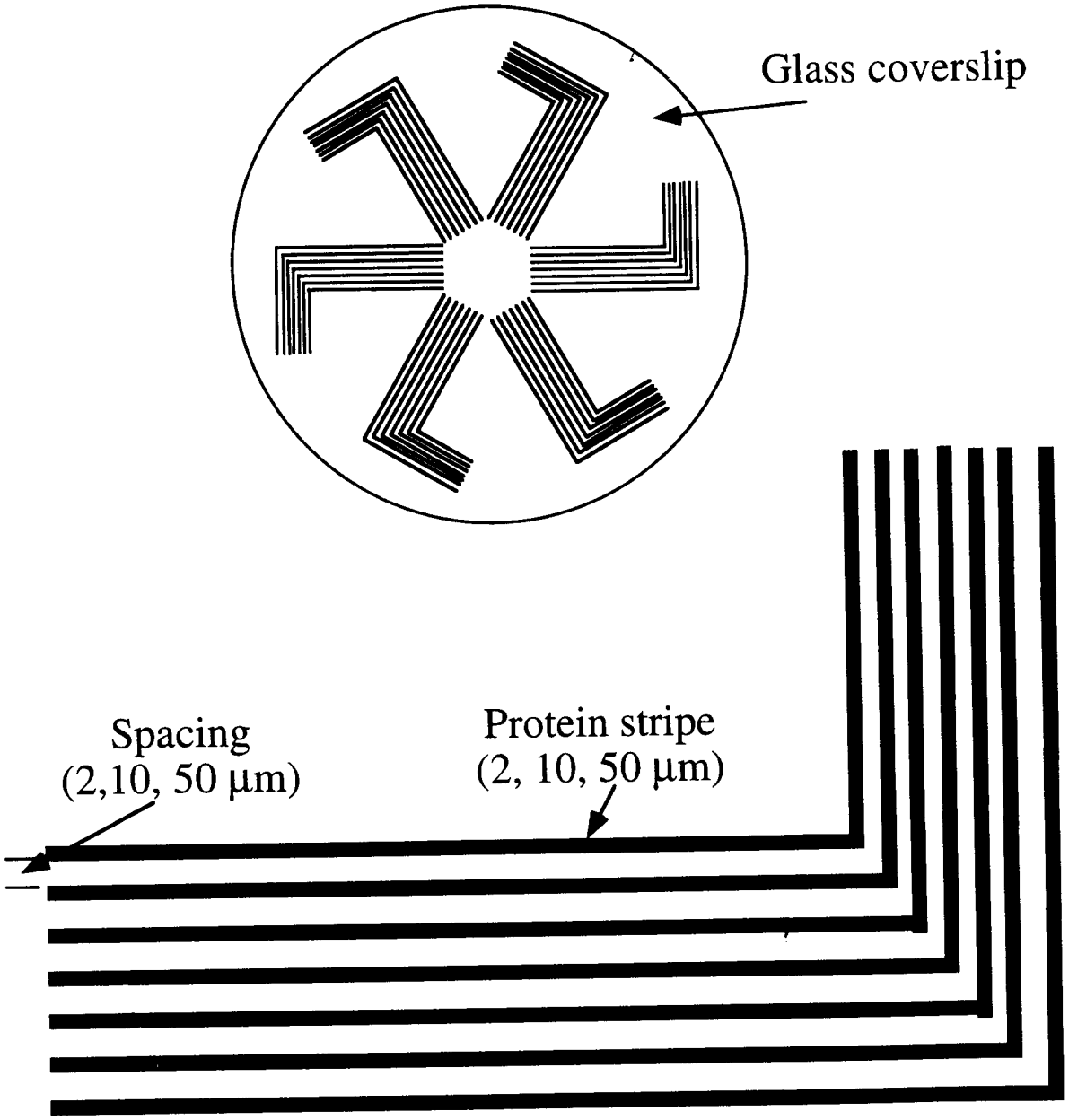
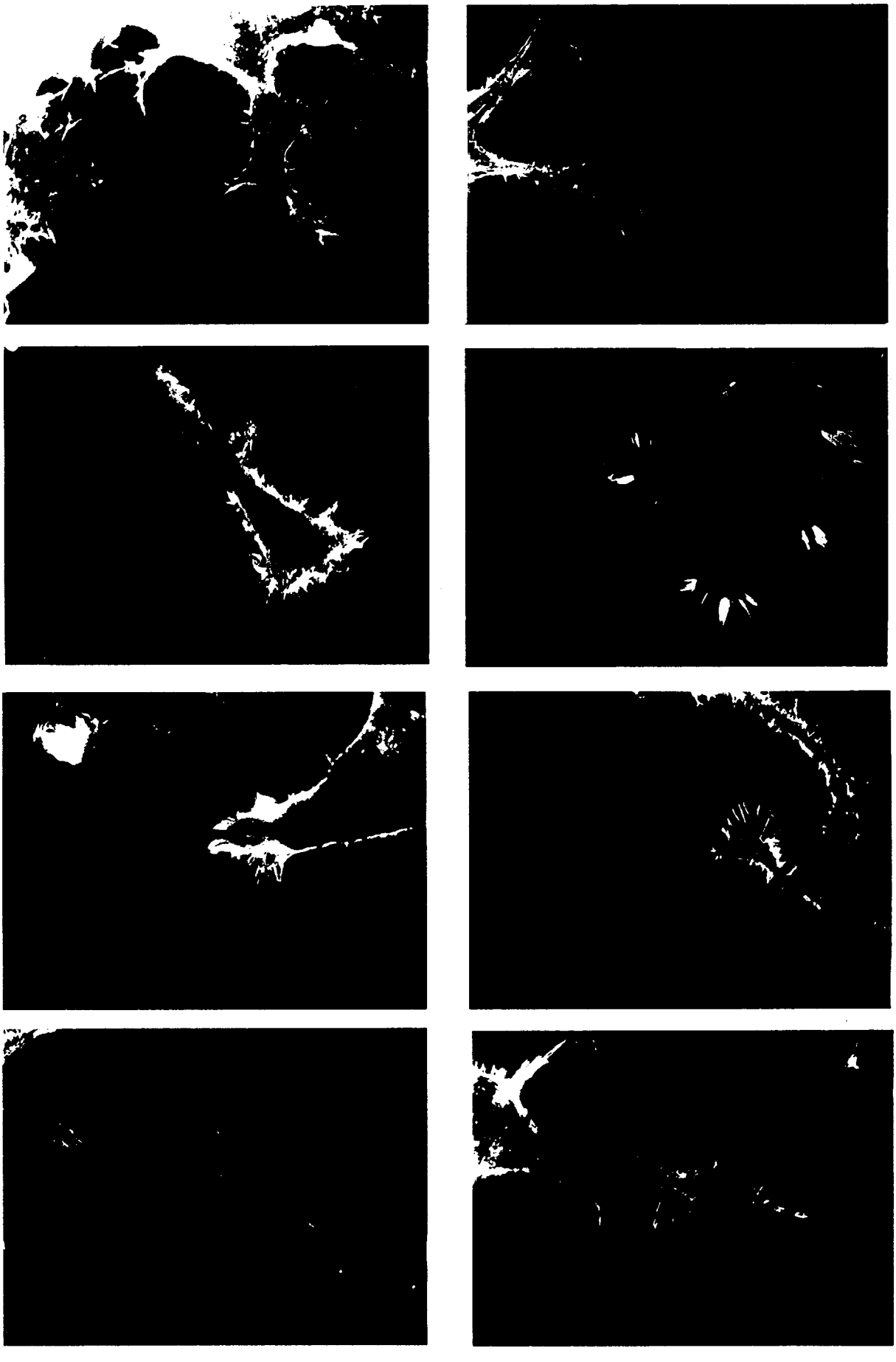
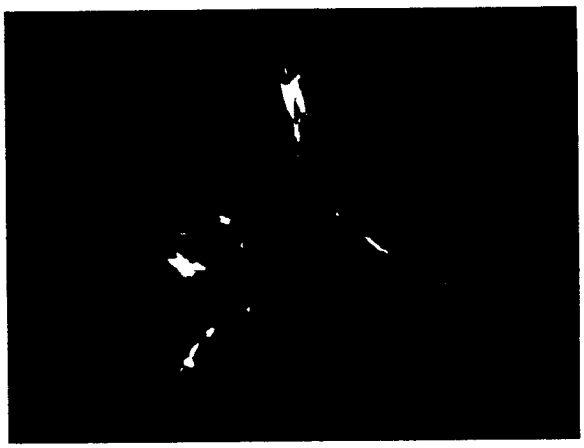
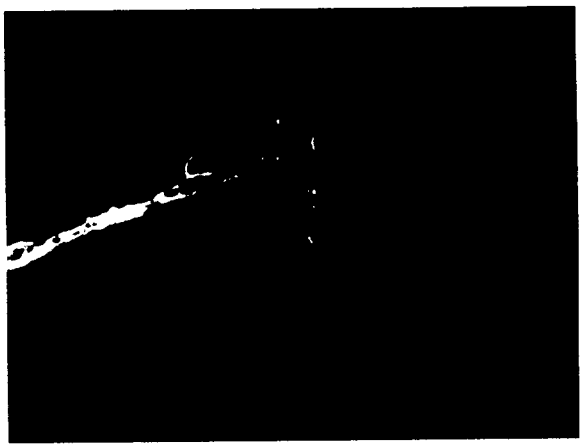





Glass coverslip
Spacing
(2,10, 50 μm)
Protein stripe
(2, 10, 50 μm)

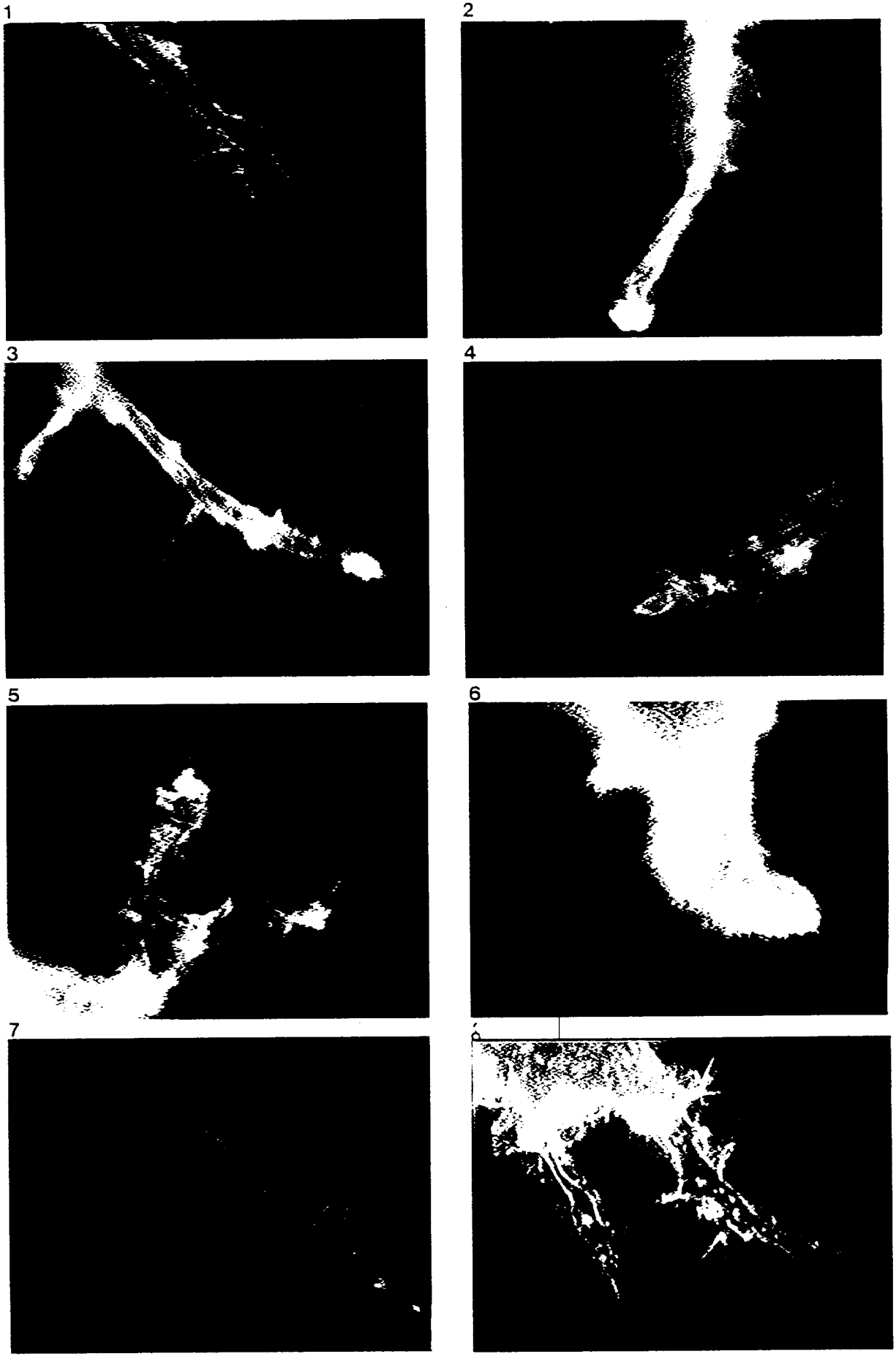


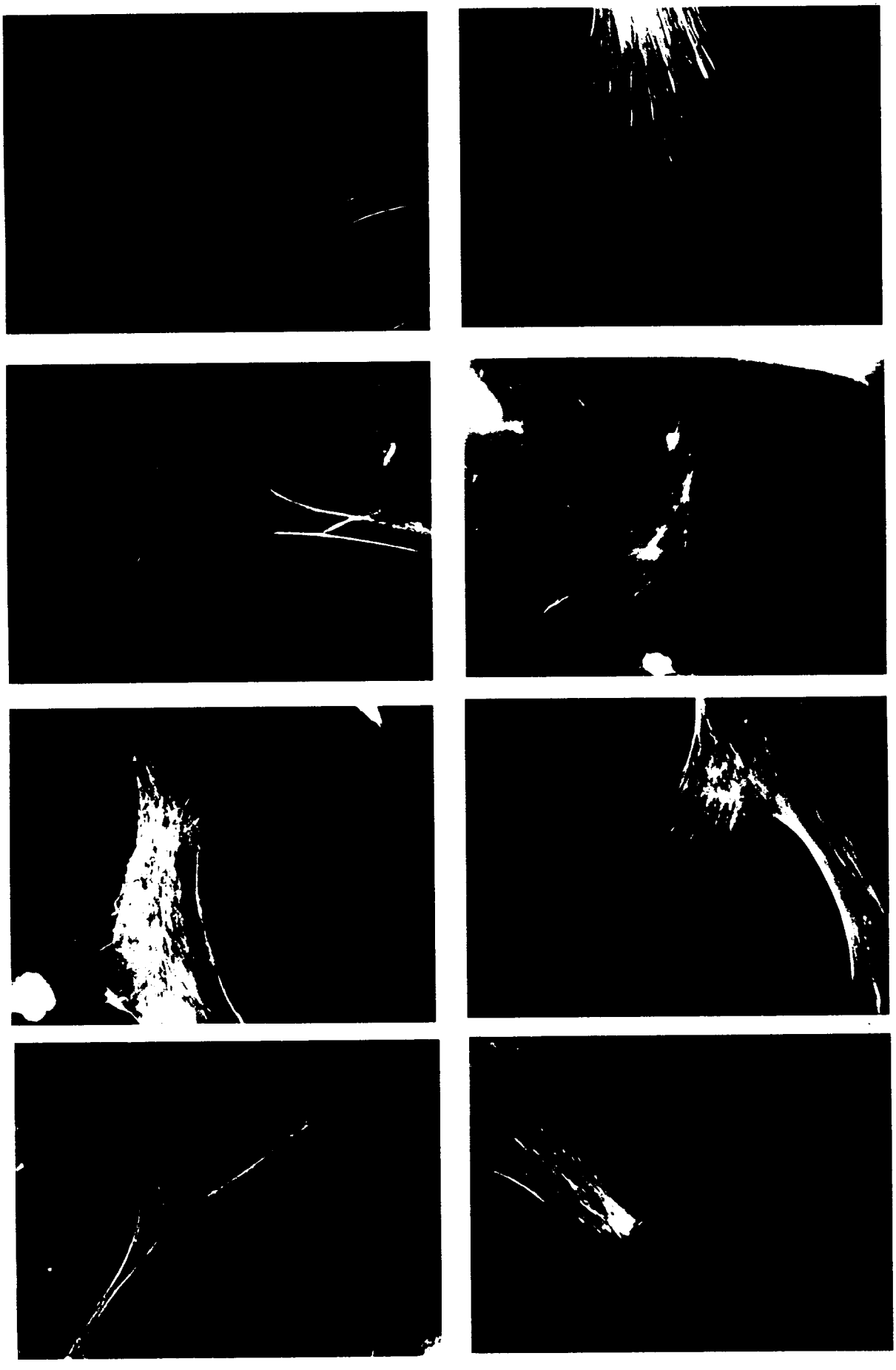




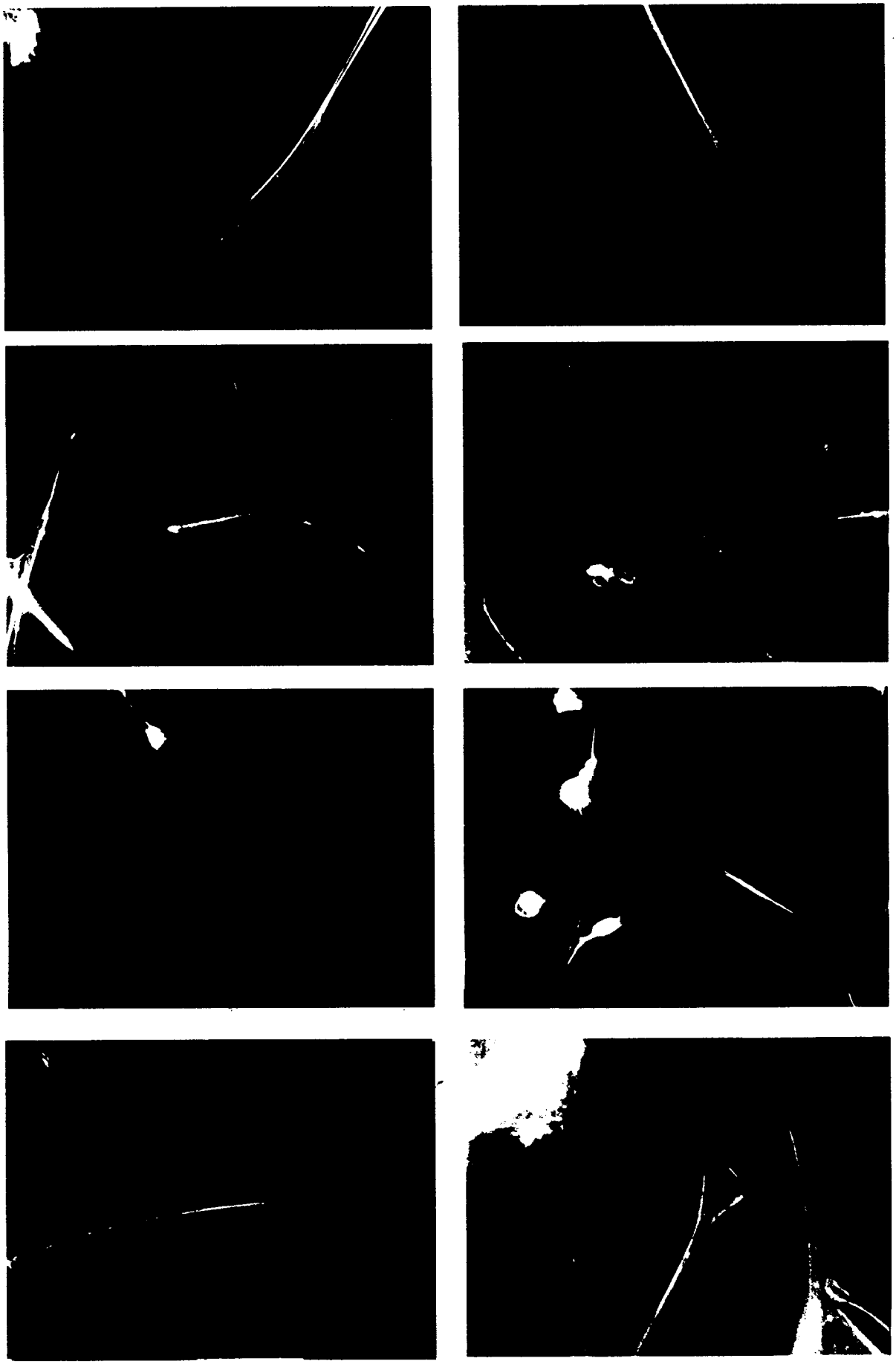
9 2A SLPL 1-4
SLPF 5-8



HO glia - FN

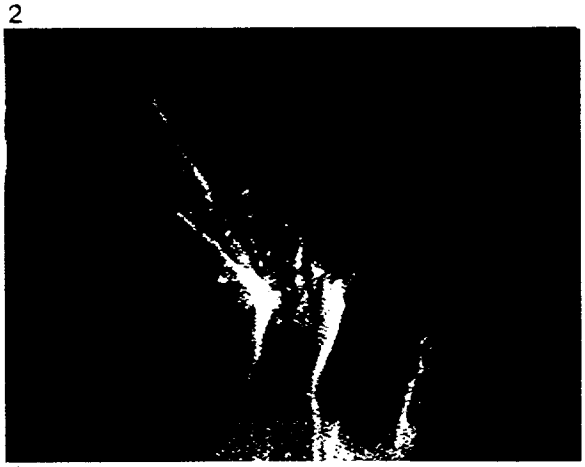


T - glia - LN



H2 glia on SCPL (1-4)
glia on SCPF (5-8)

2



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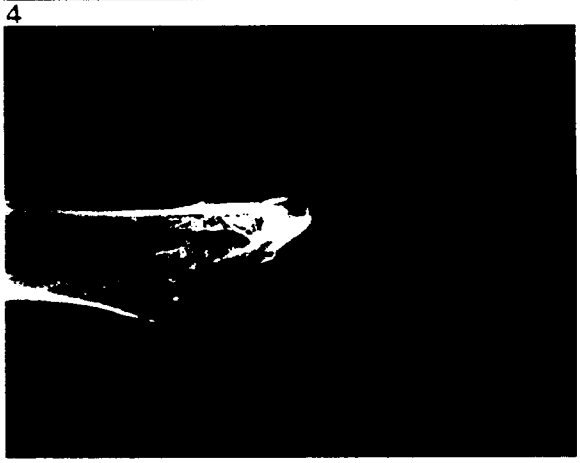

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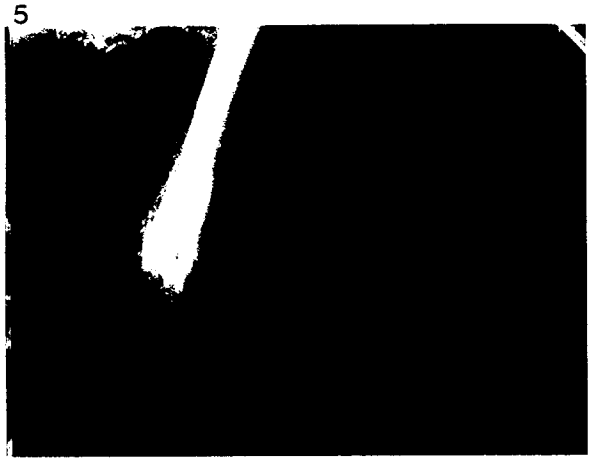

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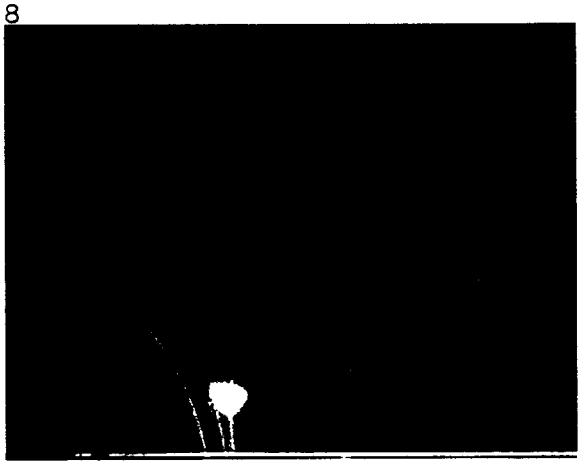
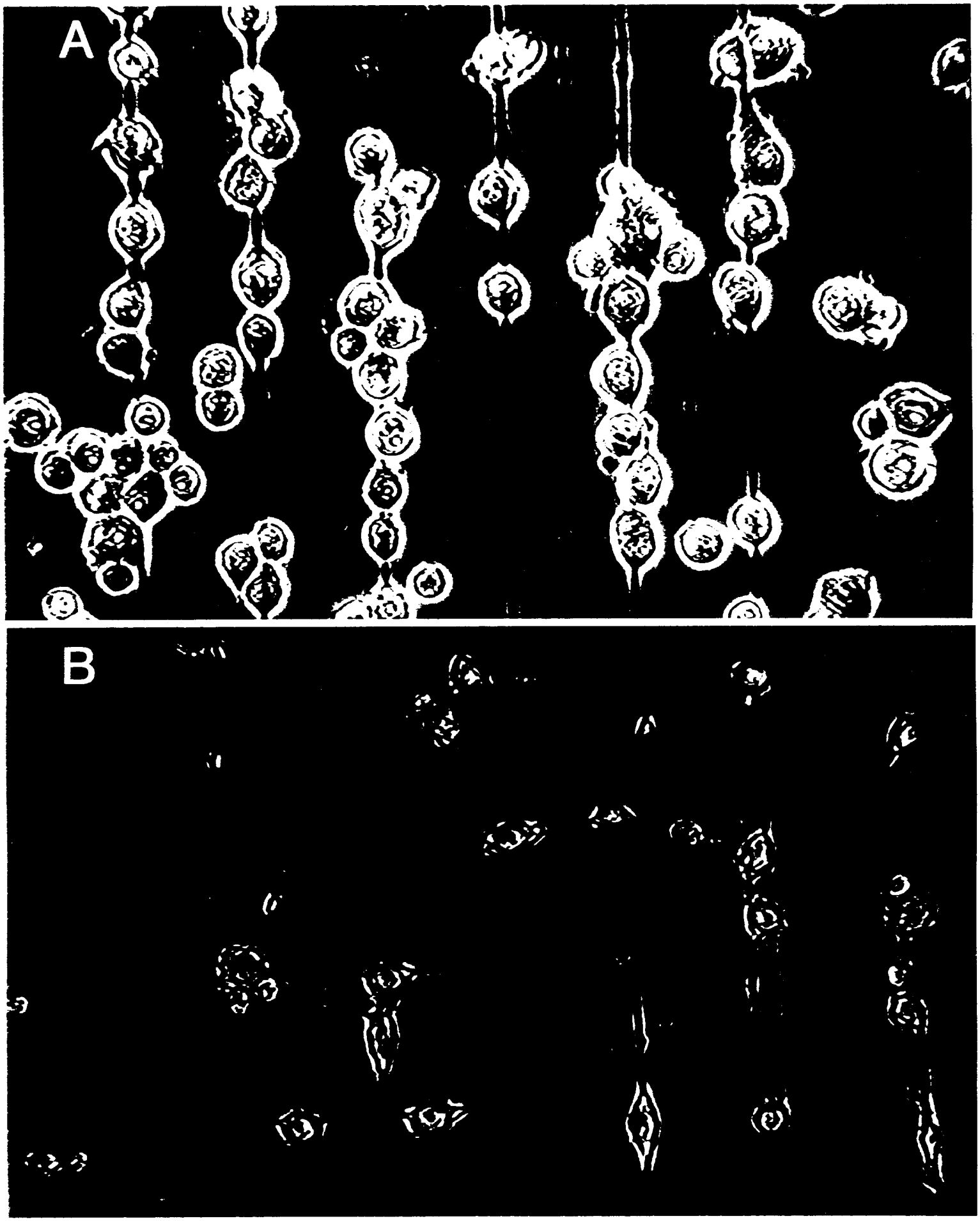
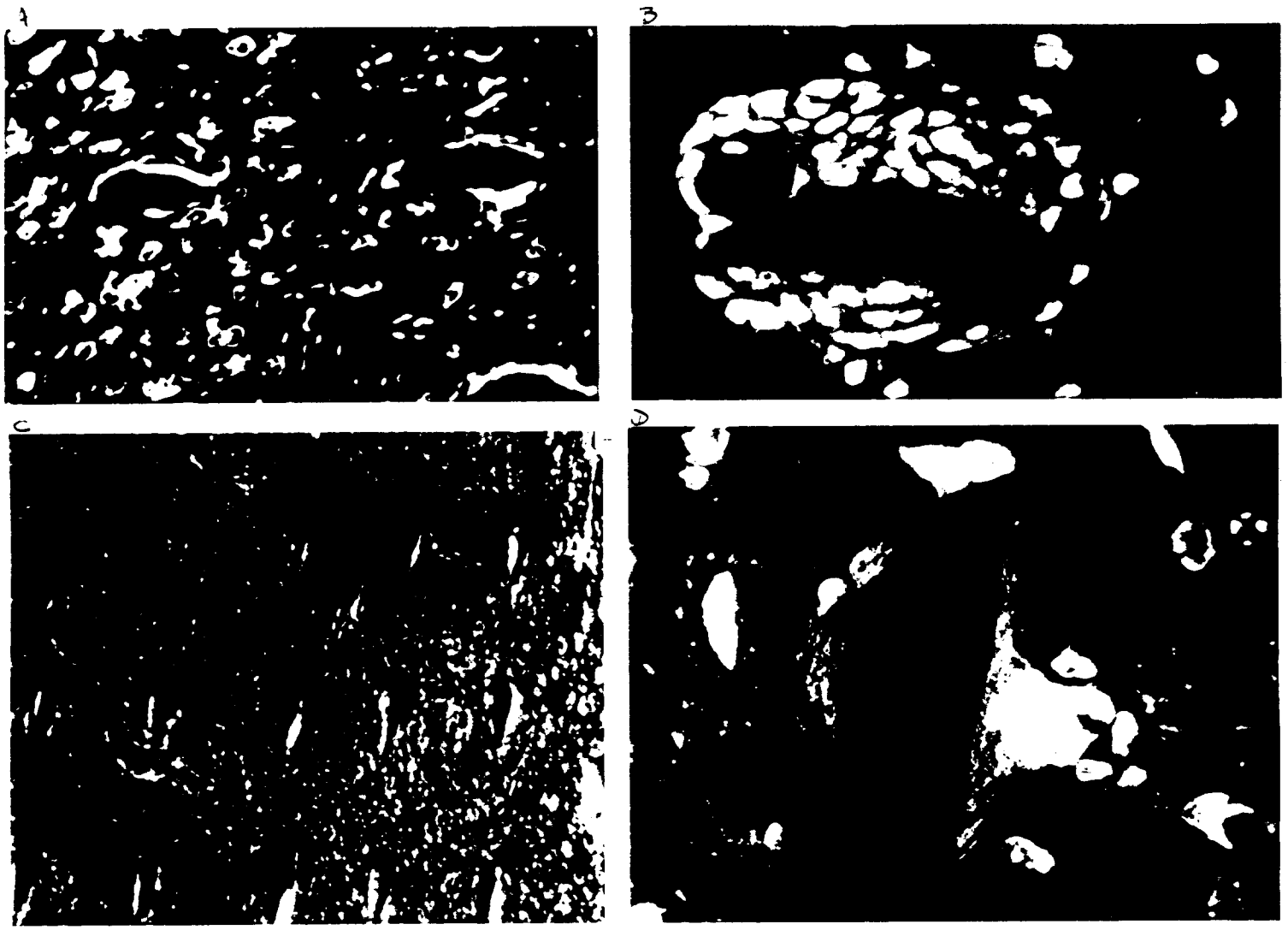



A
B


A
B
C
D